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Induction of Delayed-Type Hypersensitivity to *Leishmania major* and the Concomitant Acceleration of Disease Development in Progressive Murine Cutaneous Leishmaniasis

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BALB/c mice injected intradermally with 10^5 or higher doses of formaldehyde-fixed promastigotes (FFP) of *Leishmania major* developed strong delayed-type hypersensitivity (DTH) to leishmanial antigens injected into the hind footpad 3 to 10 days later. The DTH peaked 15 to 18 h after footpad injection and disappeared by 48 h. This specific DTH correlated with the homing of ^{51}Cr-labeled syngeneic bone marrow cells and the infiltration of proliferating cells to the site of antigen administration. Spleen cells from FFP-sensitized mice also gave significant proliferative response to FFP in vitro. The DTH was adoptively transferable by Lyt-1^+2^- L3T4^+ T cells and was *H-2* restricted. DTH could be substantially enhanced by pretreatment with cyclophosphamide or pertussigen. Such DTH enhancement was accompanied by concomitant exacerbation of disease progression after *L. major* infection. Mice injected intravenously with FFP developed substantial immunity to cutaneous leishmaniasis but specifically suppressed DTH reactivity. Treatment of mice with pertussigen before intravenous immunization, however, abolished the protection and reversed the suppression of DTH. These results therefore demonstrate that the early-appearing type of DTH is not involved in host protection but that it actually facilitates disease progression in cutaneous leishmaniasis. Further evidence, which also shows the nonspecific nature of this disease exacerbation, is provided by local cell transfer experiments. Splenic T cells from mice sensitized to keyhole limpet hemocyanin or FFP induced significantly larger lesions compared with normal T cells when they were transferred into the footpad together with specific antigen and *L. major* promastigotes.

There is now an impressive range of evidence for a causal role of cell-mediated immunity in acquired immunity to leishmaniasis. Resistant strains of mice depleted of T cells by adult thymectomy followed by irradiation and bone marrow reconstitution are less able to control *Leishmania major* infection (20), while athymic mutants of the highly resistant CBA and C57BL/6 mouse strains are totally unable to control *L. major* infection which progresses and visceralizes (17). Normal resistance, however, can be fully restored by reconstituting these T-cell-deficient mutants with syngeneic T cells. Acquired resistance against *L. major* (21) and *L. donovani* (22) as a result of recovery from infection or prophylactic immunization (11) in resistant or susceptible mice can be adoptively transferred by T cells but not B cells (13, 15).

Cell-mediated immunity is defined as immunological responses that can be adoptively transferred by cells in which the effector stage involves direct cellular interaction. A number of well-characterized immunological phenomena belong to this category including cytotoxic T-cell reactivity, T-cell-mediated macrophage activation, and delayed-type hypersensitivity (DTH). Cytotoxic T cells may play a role in acquired immunity against leishmanial infection (3), but convincing evidence for this mechanism is so far not available. Evidence for an important role of T-cell-mediated macrophage leishmanicidal activity is now accumulating. However, the part played by DTH, which also involves specific T-cell activation of macrophages, in leishmaniasis is controversial.

Earlier studies indicated a good correlation between DTH reactivity and resistance to clinical (26) and experimental (10, 16, 21) cutaneous leishmaniasis, but more recent evidence suggests otherwise. Genetically susceptible BALB/c mice immunized intravenously (i.v.) with lethally irradiated or formaldehyde-fixed promastigotes (FFP) develop substantial resistance to *L. major* infection. These protected mice also produce antigen-specific T suppressor cells which inhibit the induction of DTH when the mice are subsequently challenged intradermally (i.d) with killed parasites (8). In contrast, mice injected subcutaneously (s.c.) or i.d. with killed promastigotes produce strong DTH. However, these percutaneously immunized mice not only fail to develop protective immunity, they invariably show exacerbated disease upon a challenge infection (14). Similar enhancement of disease development can also be achieved with T-cell lines derived from mice injected s.c. with killed promastigotes. The cloned T cells mediate DTH, help antibody synthesis, and activate macrophages for in vitro leishmanicidal activity (25).

In view of its controversial role in leishmaniasis, we began a systemic study of DTH to leishmania antigens. In this paper, we report the characterization of the DTH and provide direct evidence that the early-appearing type of DTH is not only dissociable from protective immunity but

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also can facilitate the development of cutaneous leishmaniasis.

MATERIALS AND METHODS

Mice. BALB/c mice of both sexes aged 7 to 10 weeks were obtained from an inbred colony maintained at The Wellcome Research Laboratories, Beckenham, Kent, United Kingdom. BALB.B and BALB.K congeneic mice were obtained from Olac Ltd., Bicester, United Kingdom.

Parasites. *L. major* PLV39 was maintained by continuous passages in BALB/c mice. For preparation of promastigotes, the parasites were grown in Schneider drosophila medium (GIBCO Laboratories, Grand Island, N.Y.) supplemented with 30% fetal calf serum (Flow Laboratories, Inc., McLean, Va.). The parasite cultures were expanded by passage into fresh medium every 4 to 5 days, harvested by centrifugation, washed once with saline, and counted in a hemacytometer after dilution in Lugol iodine. Formaldehyde-fixed parasites (FFP) were prepared by incubating parasites in 0.5% formaldehyde (BDH Chemicals, Poole, United Kingdom) in saline at a concentration of 2×10^8 parasites per ml for 5 min at room temperature. After incubation, parasites were washed three times in saline, recounted, and stored at 4°C at a concentration of 2×10^8/ml until use.

Mice were infected by injecting 0.1 ml of the appropriate concentration of live promastigotes subcutaneously into their shaved rumps. The lesions that developed were measured at intervals up to 120 days with a direct-reading caliper gauge (GMH-390-T; Gallenkamp, London, United Kingdom) in two perpendicular diameters. The average diameter (millimeters) was recorded and corrected by subtracting the thickness of the skin at the same site of an uninfected mouse. Results were expressed as group mean ± standard error.

Induction, elicitation, and measurement of DTH. DTH was induced by injecting each flank i.d. with FFP in 0.05 ml of saline and elicited 3 to 10 days later with FFP in 0.05 ml of saline injected into the left hind footpad. DTH was estimated by footpad swelling, bone marrow cell homing, or radioactivity accumulation as detailed below. Preliminary experiments had established that the minimum dose of antigen needed for effective DTH induction was 10^5 FFP and that the minimum dose for the elicitation of DTH was 10^6 FFP. DTH is evident on day 3 after immunization and is still detectable on day 10. For routine experiments, mice were immunized with 2×10^7 FFP, and DTH was elicited 6 days later with 10^7 FFP. The DTH thus induced cross-reacts with *L. mexicana* and *L. donovani* but not with *Trypanosoma cruzi*, sheep erythrocytes, or keyhole limpet hemocyanin (KLH).

(i) Footpad swelling. Footpad swelling was measured with a dial caliper (Pocotest, reverse spring-loaded caliper) and expressed as 24-h footpad thickness increase in 10^{-2} mm, after subtracting the background owing to antigen alone.

(ii) Bone marrow cell homing. Bone marrow cells were collected from the femurs of syngeneic mice and washed twice in RPMI 1640 medium at room temperature. They were then resuspended at 10^8 cells per ml and incubated with ^{51}Cr (100 μCi/ml; 5 mCi/10.2 μg of Cr; Radiochemical Centre, Amersham, United Kingdom) for 30 min at 37°C. The labeled cells were washed three times in RPMI 1640, resuspended to 5×10^7 cells per ml, and injected i.v. (10^7 cells per mouse) 6 h after DTH elicitation. Mice were sacrificed 24 h after footpad injection, and the injected footpad, together with the contralateral uninjected footpad, were measured for thickness and cut off below the joint. Radioactivity in the footpad was measured with an LKB Wallac 1260 multigamma counter (Bromma, Sweden) and expressed as counts per minute ± standard error of the mean.

(iii) Radioactivity accumulation. Twenty-four hours before DTH elicitation, mice were injected intraperitoneally (i.p.) with 0.1 ml of freshly prepared 1.0 mM 5-fluorodeoxyuridine (Sigma Chemical Co., St. Louis, Mo.) followed 30 min later with 2 μCi (0.2 ml, i.p.) of radiolabeled (^{125}I) 5-iodo-2-deoxyuridine solution (74 kBq, 5 Ci/mg; Radiochemical Centre). All reagents were diluted in sterile saline immediately before use. At 24 h after footpad injection, mice were sacrificed, footpad swelling was measured, the footpad was removed, and radioactivity was measured in a gamma spectrophotometer. The radioactivity accumulated in the footpad was expressed as counts per minute ± standard error of the mean.

Histological examination. Footpads were removed at 15 to 18 h after DTH elicitation and stored in 10% Formalin. They were fixed, sectioned in paraffin, and stained with hematoxylin and eosin.

Lymphocyte proliferation assay. Spleens were collected in culture medium (RPMI 1640 supplemented with 10% fetal calf serum, glutamine, penicillin, streptomycin, and 20 μM 2-mercaptoethanol). A single-cell suspension was made by homogenizing spleens and passing the resulting suspension through a fine stainless-steel sieve. The cells were then washed, and viability was estimated by trypan blue dye exclusion. Cultures were set up in 96-well round-bottom microtiter plates (Titertek) with each well containing 10^5 cells in 0.2 ml of medium. Cells were stimulated with phytohemagglutinin (phytohemagglutinin HA16, 10 μg per well; Wellcome Research Laboratories) or FFP (10^5 organisms per well), or left untreated. After 92 h, cultures were pulsed with 1 μCi of [^{3}H]thymidine (24 Ci/mM; Radiochemical Centre) and harvested 4 h later with an automated cell harvester (Titertak Skatron, Skatron A/S). Radioactivity incorporation was measured in a beta-counter (LKB Wallac 1215 Rackbeta liquid scintillation counter), and the results were expressed as the mean of six replicates ± standard error of the mean.

Adoptive transfer of DTH. A total of 10^8 spleen cells from immunized or normal donors were rountinely transferred i.v. into normal recipients which were injected immediately in the hind footpad with 10^7 FFP for DTH elicitation. In some experiments, 2×10^6 immunoglobulin-negative (Ig^-) spleen cells were injected locally with various antigens into the hind footpad, and the footpad swelling was measured 24 h later.

Treatment of cells to determine cell surface phenotype. A total of 10^8 cells per ml were treated with the various antisera as follows: Thy-1.2 (F7D5, monoclonal immunoglobulin M [IgM] antibody; 1:500 dilution; Olac Ltd.); Lyt-1.2, Lyt-2.2 (monoclonal IgG2b and IgM; 1:50 and 1:25 dilutions, respectively; Cedarlane Laboratories, Ltd.); and GK 1.5 (monoclonal IgG2b, 1:30 dilution) (9). After incubation for 30 min at room temperature, the cells were sedimented by centrifugation and incubated for 45 min at 37° with a 1:10 dilution of complement (Low-Tox-M rabbit complement; Cedarlane Laboratories, Ltd.) that had been previously absorbed with normal mouse spleen cells for 1 h at 4°C. The cells were then washed and their viability was estimated and made up to the original cell concentration.

Anti-IgG column fractionation of spleen cells. T-cell-enriched fractions were prepared by the method described by Shand (24). This procedure reduced the Ig^+ cells from 40

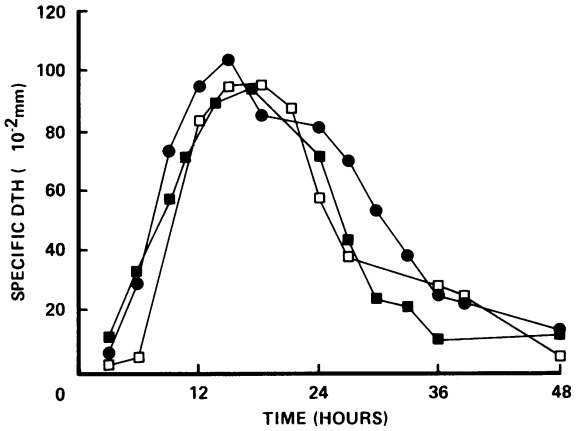

FIG. 1. Kinetics of footpad swelling. Mice were immunized with 2×10^7 FFP and injected in the footpad with 1×10^7 FFP 6 days later. Unimmunized mice were also injected in the footpad to determine background footpad reaction. Specific footpad swelling = experimental − background. The figure shows the results of three separate experiments of five mice each.

to 50% to <0.5% as assessed by an indirect immunofluorescence assay.

Treatment of mice with cyclophosphamide or pertussigen. Mice were injected with 200 mg of cyclophosphamide (cyclophosphamide monohydrate; Koch Light Laboratories, Ltd.) per kg of body weight or 400 ng of pertussigen in 0.2 ml of phosphate-buffered saline 2 days before sensitization. The crystalline preparation of pertussigen was made from the supernatant fluid of 5-day-old cultures of *Bordetella pertussis* based on the method described by Cowell et al. (7) and was a kind gift of W. A. Sewell of the Imperial Cancer Research Fund Laboratories, Lincoln's Inn Fields, London.

Statistical analysis. All experiments were performed two to four times, and representative results are shown. Statistical significance ($P < 0.05$) was analyzed by Student's t test.

RESULTS

DTH to *L. major*. BALB/c mice injected i.d. with more than 10^5 FFP developed high levels of specific DTH 3 to 10 days later when tested in the footpad with doses of FFP in excess of 10^6 organisms. The characteristics of this DTH reactivity are described in the following sections.

Kinetics of footpad swelling. Groups of mice were immunized i.d. in the flanks with 2×10^7 FFP and injected in the left hind footpad with 10^7 FFP 6 days later. The footpad swelling was determined every 3 h over a 48-h period. A significant increase in footpad thickness was first observed 6 h after antigen adminstration. This rose sharply until peaking at 15 to 18 h, and thereafter it fell steadily until it was barely detectable at 48 h (Fig. 1). Histological examination of dermis sections of the footpad at 15 to 24 h showed intense infiltration of polymorphonuclear cells and a low percentage of mononuclear cells (data not shown).

TABLE 1. Measurement of DTH

Mice[a]	Method of measurement		
	Footpad swelling (10^{-2} mm ± SE)	^{51}Cr-labeled bone marrow cell homing (cpm ± SE)	Radioisotope accumulation in footpad (cpm ± SE)
Expt	54 ± 4[b]	1,010 ± 116[b]	
Control	4 ± 2	107 ± 3	
Expt	78 ± 3[b]		3,377 ± 264[b] †
Control	4 ± 1		882 ± 84

[a] Experimental mice were sensitized for DTH with 2×10^7 FFP. Controls were left untreated. All mice were injected in the left hind footpad with 10^7 FFP. The right footpad was injected with saline and had counts per minute similar to those of the controls.

[b] $P < 0.005$.

TABLE 2. Lymphocyte proliferation assay

Cells	Antigens (cpm ± SE)		
	Phyto-hemagglutinin	FFP	None
FFP immunized	10,801 ± 422	14,010 ± 640	2,663 ± 179
Normal	8,998 ± 273	825 ± 73	397 ± 40

Measurement of DTH. Although DTH was routinely measured by footpad swelling, other methods were also used for comparison. These included the homing of ^{51}Cr-labeled syngeneic bond marrow cells to the site of antigen administration as well as the accumulation of proliferative cells in the antigen-injected footpad. All three methods recorded highly significant reactivity in the immunized group compared with controls (Table 1). The sensitivity of the three assays also appeared to be comparable, with a P value at <0.005. Since the footpad-swelling method is the simplest to perform, all subsequent DTH determinations were carried out by this measurement.

Lymphocyte proliferation assay. Although DTH was routinely measured in vivo, the lymphocyte proliferation assay, which is an in vitro correlate of T-cell activation, was also used to confirm the results obtained. Spleen cells from normal or FFP-immunized mice were stimulated in vitro with FFP or phytohemagglutinin. While both populations of cells responded to phytohemagglutinin, only cells from immunized donors proliferated significantly in response to FFP (Table 2).

Adoptive transfer of DTH. Donor mice were immunized i.d. with 2×10^7 FFP. Serum and spleen cells were collected 4 days later and transferred i.v. into normal syngeneic recipients which were tested for DTH reactivity immediately after transfer. Significant DTH was transferred systemically

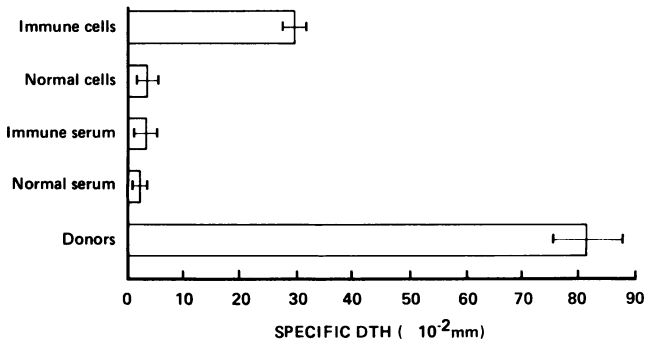

FIG. 2. Adoptive transfer of DTH. Donor mice were immunized with 2×10^7 FFP, and serum and spleen cells were collected 4 days later. Serum (0.5 ml) or cells (10^8) were transferred i.v. into normal syngeneic recipients which were tested for DTH against FFP immediately after transfer. Specific DTH = experimental − background (mean ± standard error, $n = 5$).

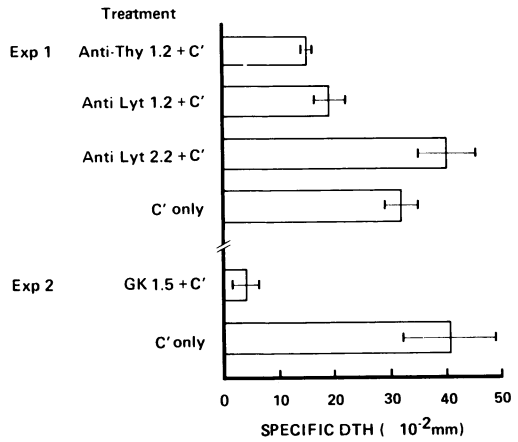

FIG. 3. Cell surface phenotype of T cell mediating DTH. Spleen cells from FFP-immunized mice were treated with antibody and complement as indicated before being transferred i.v. into normal syngeneic recipients. Vertical bars represent standard error of the mean (n = 5.)

by immune cells but not by immune serum (Fig. 2). DTH in the recipients was 30 to 50% of that observed in the donors. DTH could also be transferred locally to the footpad with 2 $\times$ 10^7 immune spleen cells mixed with 10^7 FFP (data not shown).

Phenotype of cells mediating DTH. Spleen cells from donor mice immunized i.d. with FFP were treated with anti-Thy-1.2 plus C1, anti-Lyt-1.2 plus C1, anti-Lyt-2.2 plus C1, GK 1.5 plus C1, or C1 alone. The DTH-mediating potential of the spleen cells was significantly reduced by treatment with anti-Thy-1.2 plus C1, anti-Lyt-1.2 plus C1, and GK 1.5 plus C1 but not with anti-Lyt-2.2 plus C1 (Fig. 3). Therefore, DTH was mediated by Thy-1^+ Lyt-1^+2^- L3T4^+ cells.

***H-2* restriction of DTH.** Reciprocal cell transfer experiments with *H-2* congeneic mice of BALB background (BALB.B and BALB.K) were done to determine the *H-2* restriction on the T-cell-mediating DTH and to further exclude the possible involvement of antibody. Results (Fig. 4) demonstrate that DTH could only be successfully transferred to syngeneic recipients. Spleen cells from immune BALB/c (*H-2*^{d}) mice expressed strong DTH in syngeneic mice but not in BALB.B (*H-2*^{b}) or BALB.K (*H-2*^{k}) mice. Conversely, spleen cells from BALB.B or BALB.K donors could only transfer DTH to BALB.B or BALB.K recipients, respectively, but not to BALB/c mice.

Enhancement of DTH and its effect on the course of *L. major* infection. After establishing and characterizing the DTH reactivity in the present system, we investigated the effect of DTH on *L. major* infection in susceptible BALB/c mice. Cyclophosphamide and pertussigen were used to enhance DTH since they have been shown in several experimental models to greatly increase and sustain DTH reactivity. Groups of mice were injected i.p. with 200 mg of cyclophosphamide per kg or 400 ng of pertussigen, immunized i.d. 2 days later with 2 $\times$ 10^7 FFP, and infected 6 days later with 2 $\times$ 10^5 *L. major* promastigotes. Treatment of mice with cyclophosphamide or pertussigen substantially enhanced the DTH to FFP compared with that of untreated but immunized and infected mice (Fig. 5 and 6). The lesion development was also significantly accelerated in both cyclophosphamide- and pertussigen-treated mice compared with untreated controls. These results indicate that enhancement of DTH, particularly early in infection, is associated with an exacerbation of the disease. The DTH induced by FFP in cyclophosphamide- or pertussigen-treated mice has the same characteristics of kinetics and cellular infiltrate as the DTH sensitized by FFP alone (data not shown).

Reversal of protective immunity and restoration of DTH by pertussigen. Early studies have shown that BALB/c mice injected i.v. with 4 $\times$ 10^7 FFP develop profound specific suppression of DTH to FFP and a concomitant immunity to a challenge infection of *L. major* promastigotes (8). Experiments were therefore done to investigate the effect of pertussigen on DTH and protection after i.v. immunization with FFP. Groups of mice were immunized i.v. with 4 $\times$ 10^7 FFP with or without pertussigen pretreatment and were infected s.c. with 2 $\times$ 10^5 *L. major* 6 days later. Immunized mice not pretreated with pertussigen showed significant delay in the onset of lesion development as compared with unimmunized controls. However, such protection was substantially reduced by pretreatment with pertussigen (Fig. 7). Mice receiving i.v. doses of FFP developed markedly lower levels of DTH for at least 70 days after infection as compared with unimmunized controls. This suppression was completely eliminated by treatment with pertussigen (Fig. 8).

Local transfer of DTH and exacerbation of disease progression. Spleen cells from donor BALB/c mice immunized i.d. with 20 μg of KLH or 2 $\times$ 10^7 FFP 6 days previously were enriched for Ig^- (T) cells with anti-immunoglobulin columns. A total of 2 $\times$ 10^6 Ig^- cells were injected into the footpads of BALB/c mice together with 25 μg of KLH or 2 $\times$ 10^6 FFP and 2 $\times$ 10^5 *L. major* promastigotes. Significant footpad swelling was observed in mice receiving KLH or FFP immune cells but not normal cells (Table 3). This DTH subsided by 48 h, and no increase in footpad thickness was detected until around 40 days after infection. Thereafter, the

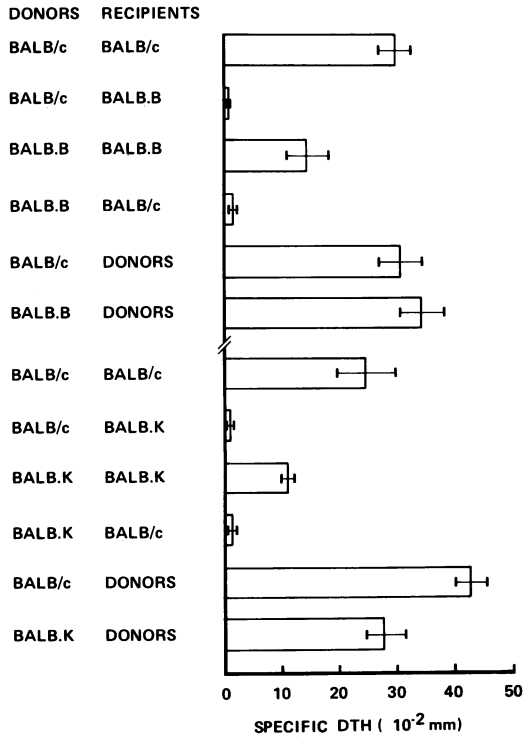

FIG. 4. *H-2* restriction of DTH to FFP. For details of cell transfer see the legend to Fig. 2. Vertical bars show the standard error of the mean (n = 5).

infected footpad became inflamed, and the disease developed rapidly, particularly in those mice receiving immune cells. These mice showed greatly accelerated disease progression compared with recipients of normal cells (Table 3). It is significant that mice receiving KLH-primed cells also developed similarly exacerbated disease, indicating the nonspecific nature of this effect of DTH.

DISCUSSION

Cutaneous DTH is a measurement of cellular activity involving a series of interacting T cells and phagocytes. The principal characteristic of DTH is the period of latency, taking 4 to 6 h to appear and becoming fully manifest by 18 to 24 h. It gradually fades and completely disappears by 72 h. The reaction is immunologically specific and is transferable by T cells whose induction and expression are restricted by products of the major histocompatibility complex. Today, DTH encompasses the classical tuberculin-type reaction, skin contact sensitivity, and the more transient and early appearing Jones-Mote reactivity (12).The tuberculin type of DTH has the distinctive infiltration of mononuclear cells, whereas the Jones-Mote reaction in guinea pigs involves predominantly basophils rather than macrophages in the site of antigen administration. The footpad-swelling test is currently the most widely used method for detecting DTH in rodents. It generally correlates well with the bone marrow homing assay and the accumulation of proliferating cells at the site of antigen injection.

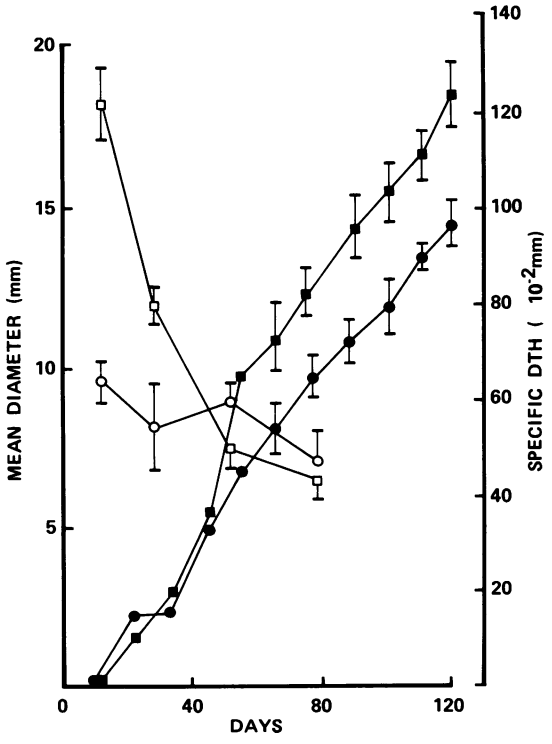

FIG. 5. Effect of cyclophosphamide on DTH and the course of *L. major* infection. Groups of 25 mice were treated with cyclophosphamide (squares) or left untreated (circles) 2 days before immunization i.d. with FFP. Six days after immunization, they were infected s.c. with 2×10^5 promastigotes. Five mice per group were used to determine the course of infection (solid symbols), while the rest were tested for DTH (open symbols) at various intervals (five mice at a time, each mouse being tested only once). Vertical bars show the standard error of the mean.

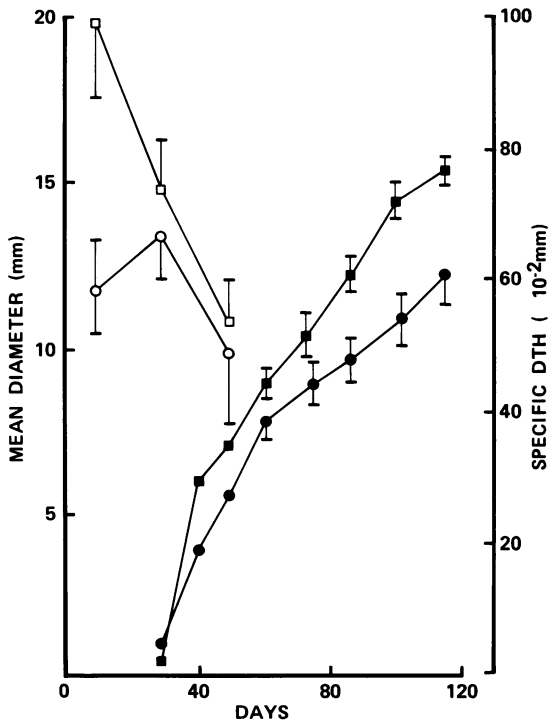

FIG. 6. Effect of pertussigen on DTH and the course of *L. major* infection. For details, see the legend to Fig. 5, using petussigen instead of cyclophosphamide.

DTH to *L. major* induced by FFP is more akin to the Jones-Mote reaction because of its early peaking kinetics and predominantly polymorphonuclear cell infiltration. It is, however, a true DTH in that the reaction is antigen specific and transferable with Lyt-1^+2^- L3T4^+ immune T cells whose expression is *H-2* restricted. Furthermore, the reactivity can be similarly demonstrated by the bone marrow homing assay and the accumulation of proliferative cells test. The immune cells also proliferate specifically to FFP in vitro.

DTH can be significantly enhanced and sustained by cyclophosphamide or pertussigen given in moderate doses shortly before immunization. Cyclophosphamide is thought to exert its DTH-enhancing activity by preferentially inhibiting suppressor-T-cell precursors (2) through direct alkylation of nuclear DNA. Pertussigen causes profound impairment of lymphocyte homing to lymph nodes and is associated with a relatively greater depletion in lymph nodes of Lyt-2^+ T cells and B cells and an accumulation of Lyt-2^- L3T4^+ T cells. It has been suggested that it is this effect together with the mitogenic action of pertussigen that contributes to enhanced DTH responses (23). BALB/c mice treated with cyclophosphamide or pertussigen before i.d. injection with FFP and s.c. infection with *L. major* promastigotes showed markedly enhanced DTH reactivity compared with untreated controls. These treated mice also developed significantly accelerated lesion progression (Fig. 5 and 6). There is thus a direct correlation between DTH reactivity and exacerbation of cutaneous leishmaniasis.

We have previously demonstrated that BALB/c mice injected i.v. with FFP develop substantial immunity to *L. major* as well as long-lasting specific suppression of DTH (8). Results obtained in the present study show that the situation can be reversed by prior treatment with pertus-

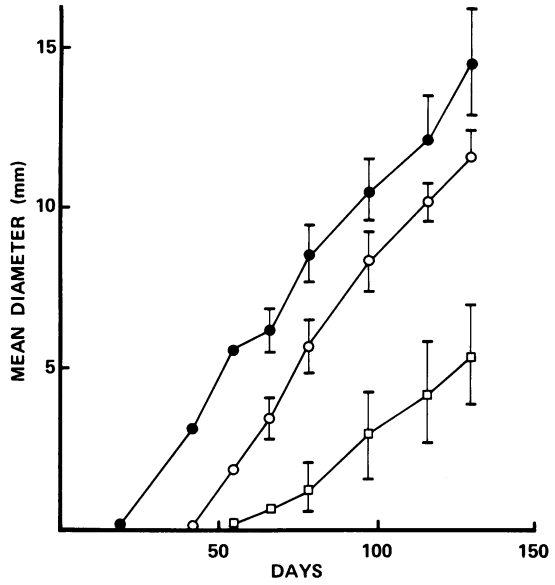

FIG. 7. Reversal of protective immunity by treatment with pertussigen. Mice were immunized i.v. with 4×10^7 FFP with (○) or without (□) pretreatment of pertussigen. Together with untreated and unimmunized controls (●), they were infected with 2×10^5 promastigotes 6 days later. Vertical bars show the standard error of the mean ($n = 5$).

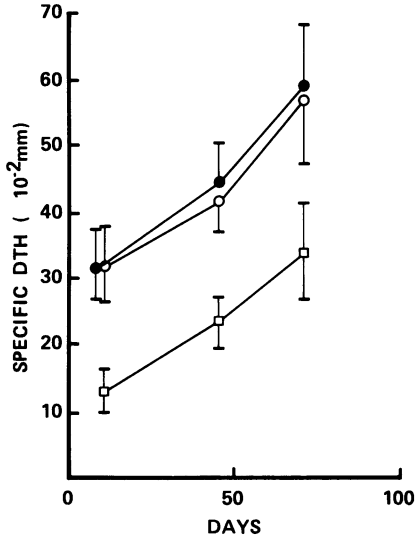

FIG. 8. Reversal of DTH suppression by treatment with pertussigen. For details and symbols see the legend to Fig. 7. Each mouse was tested for DTH only once. Vertical bars show the standard error of the mean ($n = 5$).

sigen. Thus, BALB/c mice injected i.p. with pertussigen 2 days before i.v. immunization with FFP developed normal DTH reactivity to FFP but were incapable of mounting significant resistance to *L. major* infection (Fig. 7 and 8). Collectively, these data argue strongly that DTH is in fact detrimental to the host in cutaneous leishmaniasis.

To demonstrate this point even further, T cells from FFP- or KLH-immunized mice were transferred locally together with FFP or KLH and infective *L. major* into the footpads of normal syngeneic recipients. Mice receiving the immune T cells mixed with the corresponding antigen produced local DTH reactivity and significantly exacerbated disease compared with mice given normal T cells. These results therefore provide further evidence that DTH enhances lesion development and indicate that the effector stage of the disease-promoting effect of DTH is not antigen specific since it can be equally accomplished by DTH to KLH. A similar finding was reported earlier by Titus *et al.* (25) who showed that DTH to ovalbumin can also enhance *L. major* lesion development provided the infectious promastigotes were injected locally together with ovalbumin in the footpads of BALB/c mice.

The finding that DTH has a detrimental effect in murine cutaneous leishmaniasis is in apparent contrast to the earlier observations that DTH could be a host-protective cell-mediated immunity in clinical and experimental leishmaniasis. Bryceson (4) reported the association of clinical development of diffuse cutaneous leishmaniasis and impairment of specific skin hypersensitivity. In guinea pigs, there was also a characteristic and selective depression of DTH when primary lesions were well developed and metastatic infection of *L. enrietti* was well advanced (5, 6). Furthermore, Preston et al. (19) showed that the relative susceptibility of some inbred mouse strains to *L. major* correlated directly with reduced specific DTH reactivity. Recently, too, it has been demonstrated that sublethally irradiated BALB/c mice which recovered from *L. major* infection developed high and sustained levels of specific DTH. This is in contrast to unirradiated mice whose initial DTH is specifically and strongly suppressed as the infection progresses to uniform fatality.

The following interpretation may account for the apparent discrepancy in the role of DTH in cutaneous leishmaniasis. Cutaneous DTH is a gross measurement of a number of distinct but related immunological reactions. For example, the early-appearing Jones-Mote reaction (12) and the classical late-occurring tuberculin type of hypersensitivity involve different cellular infiltrates and may be mediated by distinct lymphokines. It is clear that the DTH induced by i.d. immunization belongs to the Jones-Mote reaction, whereas the DTH resulting from convalescent immunity appears to be akin to the tuberculin type of DTH in both kinetics of appearance and histological cellular infiltration (J. S. Dhaliwal and F. Y. Liew, submitted for publication). Disease exacerbation and impairment of prophylactic i.v. im-

TABLE 3. Local transfer of DTH and disease development in the footpad

Donor cells	Antigens	24-h DTH[a] (10^{-2} mm)	Lesion size (10^{-2} mm)[a]		
			Day 30	Day 49	Day 57
KLH immunized	KLH + 2×10^5 *L. major*	35 ± 2^b	0	48 ± 19^b	108 ± 31^b
FFP immunized	FFP + 2×10^5 *L. major*	42 ± 3^b	2 ± 1	34 ± 13^b	119 ± 13^b
Normal	FFP + 2×10^5 *L. major*	3 ± 2	3 ± 0	3 ± 2	44 ± 9

[a] Difference in thickness between footpad injected with cells plus antigen and control uninjected footpad.
[b] $P < 0.005$ compared with respective normal controls (line 3).

munization by pertussigen treatment and i.d. injection may conceivably result from the induction of Lyt-1^+2^- L3T4^+ T cells, which recruit target cells that are less hostile to the invading parasites and thus allow them to multiply in vivo. The DTH resulting from convalescent immunity may attract as well as activate macrophages which are capable of limiting the replication of the invading parasites. Indeed, it now appears that T cells from mice recovered from *L. major* infection are capable of elaborating gamma interferon and macrophage-activating factor which enable infected macrophages to eliminate intracellular parasites. In contrast, T cells from mice immunized i.d. with FFP are devoid of such reactivity (Dhaliwal and Liew, submitted). This interpretation implies the functional heterogeneity of Lyt-2^- L3T4^+ T cells, a concept that is increasingly supported by recent observations (1, 18).

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